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Afforestation, Natural Secondary Forest or Dehesas? Looking for the Best Post-Abandonment Forest Management for Soil Organic Carbon Accumulation in Mediterranean Mountains

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Abstract: Forest expansion in Mediterranean mountain areas is a widespread phenomenon resulting from the abandonment of agricultural and pastoral activities during the last century. Therefore, knowledge of the long-term storage capacity of soil organic carbon (SOC) in Mediterranean forests is of great interest in the context of global change. However, the effects of these land uses and covers (natural secondary forest, afforestation with conifers and silvo-pastoral ecosystems (dehesas)) on SOC dynamics are still uncertain. The main objectives of this study were to evaluate physico-chemical soil properties, SOC and nitrogen stocks, and SOC fractions in Mediterranean forests and to assess the effects of tree species, the soil environment (acidic and alkaline), and land management. We selected five land uses and land covers: managed and non-managed afforestation and dehesa (except for alkaline dehesa) and a stage of succession when tree species begin to become established after abandonment. This study concludes that although total SOC stocks are higher in afforested systems with conifers, SOC is stored in less stable carbon pools than in broadleaf forests. In addition, this study confirms that there are marked differences in the results between acidic and alkaline environments. Finally, the management system is also a significant factor, particularly for afforested sites.

Keywords: afforestation; natural revegetation; silvo-pastoral systems; soil organic carbon fractionation; Iberian System (Spain)

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1. Introduction

Forest ecosystems can accumulate significant amounts of organic carbon in both the biomass and soil. The global forest carbon stock is estimated to be 662 Gt, of which 45% is in soil organic matter [1,2]. A net forest increment has been described by [3] in boreal and temperate zones (including the Mediterranean zones). At the European level, a mean net forest increase of 0.4 million ha/y was reported for the period 2010–2015. Spain is the country with the third largest forest area within the European Union, with approximately 26 million ha of forest ecosystems (around 50% of the country's area) [4], with *Pinus nigra*, *Pinus sylvestris* and *Quercus pyrenaica* being the most common species.

Extensive areas of agricultural and pasture land in the Mediterranean region were abandoned during the latter half of the 20th century [5–7]. As a consequence of this process, new forests due to secondary succession or active restoration (afforestation that mostly consists of tree planting, mainly conifers) have generally been established in less productive and accessible areas, resulting in a landscape of regenerated vegetation and afforestation. In addition, traditional silvo-pastoral systems (hereafter referred to as dehesas) are conserved close to villages. These consist of natural open cleared forests (mainly *Quercus* spp. trees) and are a form of land use in which trees, pastures and livestock share the same territory.

Typically established on less fertile soil, these areas primarily focus on extensive livestock raising and the production of acorns and fuelwood [8].

The intense process of revegetation (by means of afforestation and natural revegetation) has led to several negative impacts: (i) an increase in the number and intensity of wildfires [9]; (ii) the loss of cultural landscapes and biodiversity [10]; (iii) a reduction in pastures, traditional knowledge and opportunities for rural development based on extensive livestock [11,12]; and (iv) a decrease in water resources, with significant consequences, as most of the water in the Mediterranean basin is generated in the mountains [13]. Among the positive impacts, the reduction in soil erosion [6], greater soil organic carbon stocks [14,15], and the increase in species associated with forest systems should be highlighted.

Forest soils are important reservoirs of soil organic carbon (SOC), and the establishment of new forest systems in recent decades has led to changes in biomass, deadwood, litter, physico-chemical soil properties, and SOC accumulation. Various factors influence SOC stocks, including the underlying bedrock, land use history, dominant forest tree species, forest management practices, the rate of litter decomposition, and the type of organic material present (e.g., leaf decomposition from deciduous species occurs more rapidly than for conifers) [16–18].

In the Mediterranean mid-mountains, coniferous and broadleaved forests cover millions of hectares. Consequently, knowledge of the long-term storage capacity of SOC in forest soils is of great importance for the adaptation to and/or mitigation of global change. However, the effects of these forest systems (natural secondary forest, afforestation with coniferous and silvo-pastoral ecosystems (dehesas)), forest management practices, and bedrock lithology on physico-chemical soil properties and on SOC dynamics are still uncertain and are not well known. Likewise, the impact of land abandonment and land use and land cover (LULC) on soil properties in Mediterranean mountains has been widely described in environments with alkaline soils [17,19]. Yet, there is limited knowledge regarding the impacts of land abandonment in environments with acidic soils. Low soil pH plays a key role in vegetation production and SOC accumulation, together with the absence of carbonates and consequently the limited soil aggregation process. This suggests differences in soil properties and SOC storage between acidic and alkaline soil environments [17].

The stabilization of SOC in soils is important with respect to the recalcitrance against degradation of the soil organic matter. It is not just the amount of SOC that is important, but also the type of organic matter, and more importantly whether the organic carbon is protected against mineralization as well as the environmental conditions [20–22]. It is important to note whether the organic matter is easily accessible or not. The association of organic matter with minerals, or the occlusion of organic matter in soil aggregates, reduces accessibility, and therefore the soil organic matter will generally be more recalcitrant to degradation. Furthermore, the soil microbiome and the microbial interactions with the SOC soil characteristics [23], including pH and mineralogy [24,25], also play a role in the degradation. Separating the soil organic carbon into different fractions is one of the methods to determine the accessibility and recalcitrance of organic carbon in the soil. Soil carbon can be fractionated into the heavy fraction (HF), a proxy for mineral associated organic carbon, the occluded light fraction (OLF), a proxy for the occluded material, and the light fraction (FLF), a proxy for easily accessible soil carbon [26].

Our two working hypotheses are as follows: (i) in Mediterranean mid-mountains, afforestation after land abandonment can increase soil organic carbon stocks compared to natural revegetation forest (secondary forest after land abandonment) and traditional silvo-pastoral systems (old cleared forest, dehesas), and (ii) the results differ between soil environments (significant differences in soil parameters within a same land use or cover between acidic and alkaline soil environments), plant species (coniferous and broadleaved species), and land use history. The main objectives are as follows: (i) to analyze physico-chemical soil properties in Mediterranean forest systems; (ii) to quantify soil organic carbon and nitrogen stocks in different Mediterranean forest systems, (iii) to assess the effects of forest type (tree species) and land management on SOC in two soil environments (acidic and alkaline), and (iv) to analyze the stability of SOC storage through fractionation methods in different forest systems.

2. Materials and Methods

2.1. Study Area and Forest Site Selections

This study took place in the Leza Valley (La Rioja, Spain) (Figure 1), chosen as a representative area of the Mediterranean mid-mountains, due to its altitude ranging from 600 and 1800 m above sea level (a.s.l.), its very intensive use in the past for livestock and agricultural exploitation, and its extensive use since the mid-20th century, with a loss of 79% of its population, massive abandonment of agricultural space, and a sharp decrease in livestock densities [27].

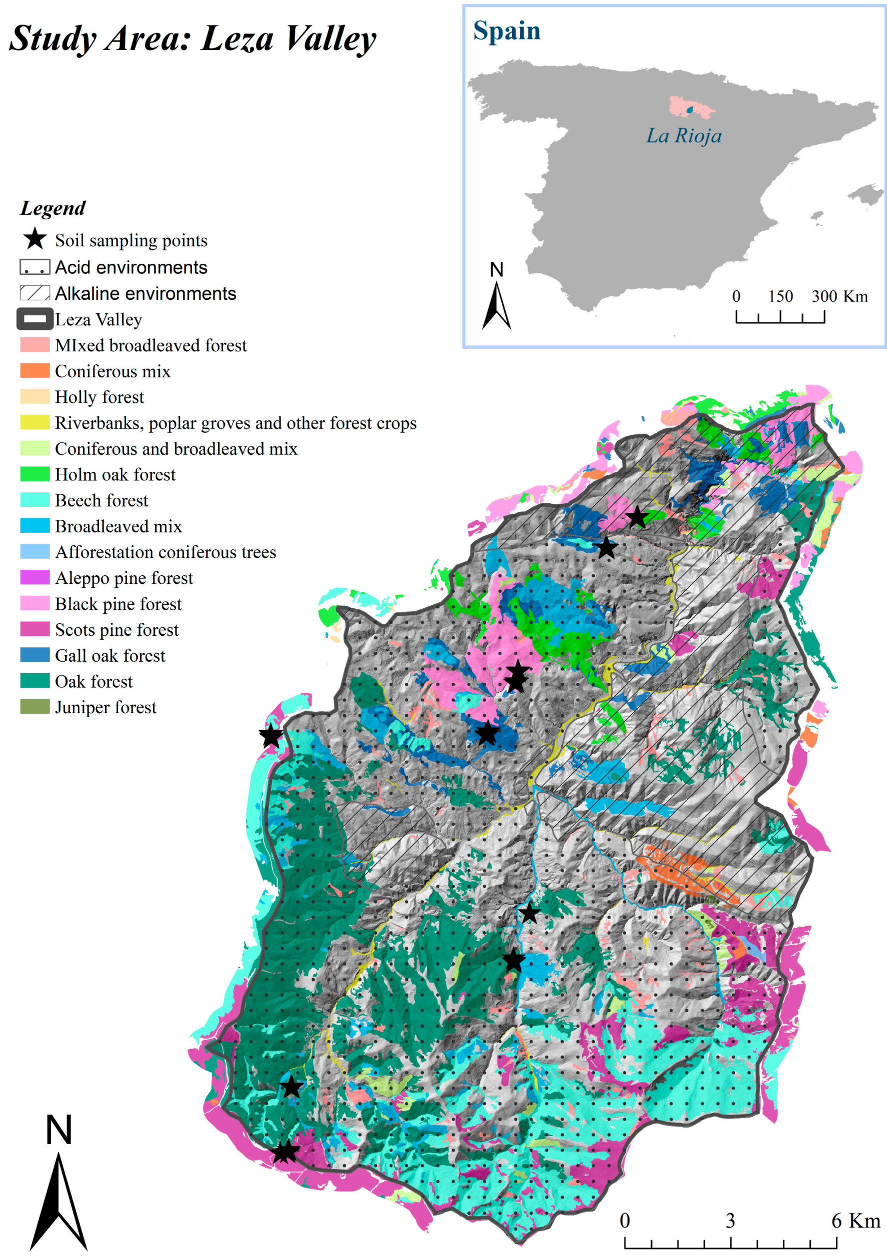

Figure 1. Forest cover in the Leza valley and location of sampling points in natural revegetated forest, dehesas and afforested sites.

The lithology of this area is mainly composed of quartzite, sandstone, and marly limestone from the Mesozoic. The relief is characterized by ridges and slopes with dominant slopes between 10 and 30%. Cuadrat and Vicente-Serrano [28] classified the climate of the study area as Mediterranean mountain. Annual rainfall varies between 600 and 1000 mm, influenced by altitude and exposure. More than 60% of the rainfall occurs in spring and autumn, while summer is characterized by dry conditions. The average temperatures are 11 °C at 600 m a.s.l. and 6 °C at 1800 m a.s.l. The soils are dominated by dystric Cambisols, which constitute well-developed soils with an acidic horizon and a high capacity to store carbon, and calcic Kastanozems of dark brown colour and high organic matter content [29]. Despite this general characterization of the lithology, it is possible to find many mixed zones between both types in the valley, a situation that poses a challenge for the location of the sampling areas.

The productive marginalization of the territory implies a process of vegetation succession, which led to the covering of the slopes by shrubs and forests (*Genista scorpius* and *Quercus faginea* in calcareous environments and *Cistus laurifolius* and *Quercus pyrenaica* in siliceous environments) [30]. Secondary succession is the most frequent process (no management) after land abandonment. It is a slow process with four stages: invasion by herbaceous plants, expansion of shrubs, entry of small trees, and stabilization of the forest stage (around 70 years old) [31].

Dehesas are multipurpose silvo-pastoral systems (old-cleared forest, around 250 years old) characterized by extensive land use, where native grasses and Mediterranean shrubs are intermixed with widely spaced and scattered Quercus [32]. The structure originates from the original forest, which is progressively cleared. In acidic soil environments, *Q. faginea* predominate, and in alkaline environments, *Q. pyrenaica* are dominant.

Afforestation has also been conducted in the context of land abandonment. In response to the gradual and slow nature of secondary succession and environmental goals aimed at regulating hydrological and geomorphic processes to mitigate flood frequency and magnitude and soil erosion, national forest services have implemented widespread afforestation programs [33]. Afforestation with coniferous trees was carried out in the second half of the 20th century at both sites after land abandonment, considering *Pinus sylvestris* (50–70 years old, with a density of 600 trees ha^{-1}) and *Pinus nigra* (50 years old, with a high density of 1000 trees h^{-1}) in both acidic and alkaline environments, respectively.

2.2. Soil Sampling and Soil Characterization

Soil sampling was conducted for five LULCs from different forest ecosystems: (i) managed afforestation (Af.M), (ii) no managed afforestation (Af.NM), (iii) managed dehesa (Dh.M), (iv) no managed dehesa (Dh.NM), and (v) a stage of succession characterized by the establishment of tree species accompanied by a well-developed undergrowth (secondary forest, F). Managed afforested areas correspond to sites where forest works have been carried out (i.e., thinning and clear-cutting harvesting where pruning remains are left on the soil). In both managed afforested and dehesa sites, extensive livestock production occurred.

Soil samples were collected from acidic and alkaline soil environments, with the exception of the samples for Dh.M, which were only obtained fromacidic environments due to the limited areas currently under management. Due to the presence of many mixed zones between soil lithologies in the general characterization of the valley, the preselection of study areas was based on a landscape characterization. Both environments were classified according to the bedrock geographical information (thanks to regional cartography [34]) and typical vegetation communities (identified from some remaining shrub species such as *Genista scorpius* in alkaline soils and *Cistus laurifolius* in acidic ones), which enabled us to differentiate the areas prior to pH and $CaCO_3$ laboratory analysis. *P. sylvestris* and *Q. pyrenaica* predominate in acidic soil environments, and *P. nigra* and *Q. faginea* in the alkaline ones according to previous vegetation study.

For each LULC category and corresponding environment, three soil samples were extracted at varying depths (0–10 cm, 10–20 cm, 20–30 cm, and 30–40 cm) following the removal of the organic layer across all plots. To capture maximum spatial variability, each sample was composed of three sub-samples. The sampling plots within each soil environment were strategically situated in areas exhibiting a comparable topography, altitude, and exposure. This approach aimed to facilitate result comparisons and minimize inaccuracies and errors.

All analyses were conducted at the laboratories of the Pyrenean Institute of Ecology (IPE-CSIC) and the Institute for Biodiversity and Ecosystem Dynamics (IBED, University of Amsterdam). The undisturbed cores underwent oven drying at 105 °C for 24 h to assess bulk density (BD). Soil samples were air-dried and sieved through a 2 mm mesh to obtain the fine fraction, from which the remaining parameters were derived. pH measurements were performed in deionized water suspension (1:2.5) using a pH meter. Texture analyses were carried out using a particle size analyzer (Mastersizer 2000, Malvern Panalytical Instruments, Almelo, The Netherlands) following the oxidation of organic matter with peroxide (H_2O_2). Total carbon (C) and total nitrogen (N) were determined using an elemental analyzer (Carbon/Nitrogen/Sulphur LECO 928 series LECO CNS 928, St Joseph, MI, USA) through dry combustion with oxygen injection. The SOC content was calculated by subtracting the % carbonate fraction from the total carbon concentrations when $CaCO_3$ was present, and was equal to the total carbon content in acidic environments. The measures of SOC and TN were reported as the concentration (%) and as the content per area (stock) expressed as inventories or stocks (Mg ha^{-1}). The SOC/N ratio was calculated from the SOC and N values.

Density fractionation was applied to bulk soil samples following the methods outlined by Golchin et al. [35] and Cerli et al. [26]. Due to the time-intensive nature of soil sample fractionation, two samples were taken for each LULC type and each depth (2 samples per 10 cm and management). After laboratory analysis, the samples were statistically grouped into two depth categories (0–20 cm and 20–40 cm) per LULC (resulting in 4 soil samples per 20 cm and LULC) to facilitate statistical analysis due to the limited number of samples. It is important to note the dataset's limitations arising from the grouping of samples at different depths when making general comments on the impact of LULCs on density fractionation results. In summary, a 10 g air-dried sample was weighed out, and 50 mL of sodium polytungstate (NaPT) with a density of 1.6 g cm^{-3} was added to a centrifuge tube. After 1 h, the suspension underwent centrifugation at 6800× g for 20 min at room temperature. The floating material (free light fraction, FLF) was separated and collected on a pore glass-fiber filter (0.7 µm Whatman GF/F filter) followed by washing with deionized water until the conductivity reached 200 µS cm^{-1}. The remaining soil was re-suspended in 50 mL of NaPT, sonicated at 150 J Ml^{-1} (Sonopuls HD 3200 with VS70 probe), and calibrated in an ice bath to maintain a temperature of 40 °C. After the dispersion process, the samples were again centrifuged at 6800× g for 20 min, filtered, and washed with deionized water as previously described (occluded light fraction, OLF). The remaining sample underwent washing through the repeated addition of deionized water, shaking and centrifugation (10,000× g to ensure complete sedimentation of the smallest clay-size particles) until the conductivity of the wash water reached 500 µS cm^{-1}. The heavy fraction (HF) was obtained with the resulting soil material. All fractions were freeze-dried, homogenized (the HF was milled), and utilized for the determination of the SOC/N ratio, SOC and N content. $CaCO_3$ content was also determined in the case of the HF, using the Bernard Calcimeter. Finally, the relative contribution of SOC by density fraction was determined by multiplying the SOC content of each fraction by its respective mass percentage.

2.3. Data Analysis and Statistical Analysis

Given that the assumption of normal distribution per factor, as assessed by the Shapiro–Wilk normality test, was not satisfied for most parameters, nonparametric tests were employed to assess differences between LULC categories. The homogeneity of variance was also examined using Levene's test. Kruskal–Wallis analyses of variance were conducted to explore differences between LULCs, soil environments and depths. Additionally, a post hoc LSD test was carried out to identify differences between LULCs at the same depth and environment. Finally, a principal component analysis (PCA) was carried out. Statistical analyses were performed using IBM SPSS Statistics 25 and RStudio (Version 2022.07.2 Build 576). Significance was considered when p values were less than 0.05 in all cases.

3. Results

3.1. Physico-Chemical Soil Properties

Table S1 in the Supplementary Materials shows the physico-chemical soil properties in the acidic and alkaline soil environments. In acidic environments, the pH was between 5.1 and 6.2, with higher values in the forest sites. In alkaline environments, the pH was higher in afforested sites, and increased with depth. In acidic environments, the BD was higher in forest sites, and significant differences ($p < 0.05$) were found at all depths between forest and afforested sites. In alkaline environments, no significant differences ($p < 0.05$) were observed in the topsoil (0–10 cm), and higher values were observed in the non-managed afforested sites (Table S1, Supplementary Materials).

Significant differences ($p < 0.05$) were found between the different forest systems related to soil texture. In acidic environments, the clay contents were significantly lower ($p < 0.05$) and the sand contents were significantly higher ($p < 0.05$) in the forest sites compared to the other LULC. No differences were observed in the silt contents in deeper soils. In alkaline environments, significant ($p < 0.05$) differences were found for all of the depths and LULC. Higher clay contents were recorded in the afforested sites (both managed and non-managed). Contrary, higher silt contents were recorded in the dehesa and forest sites (Table S1 Supplementary Materials).

The SOC contents were high in the topsoils from both sites and decreased with depth (ranging between 1.6 and 12.4 in Dh.M and Af.M, respectively, in acidic environments, and between 2.1 and 11.5 in Af.NM and Af.M, respectively, in alkaline environments). Figure 2 shows the mean values in the different LULC, including the values recorded in the organic layers, with the highest contents being recorded for both afforested sites.

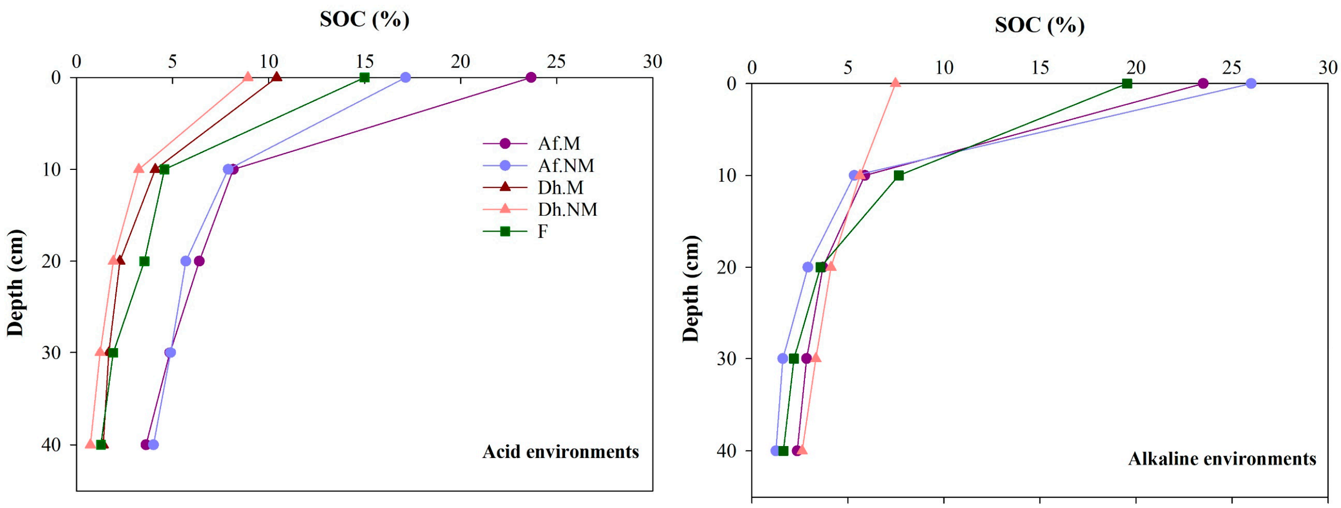

Figure 2. Soil organic carbon content, SOC (%), for the different LULC and depths in acidic and alkaline environments. Note: Af.M, managed afforestation; Af.NM, non-managed afforestation; Dh.M, managed dehesa; Dh.NM, non-managed dehesa; F, secondary forest.

In acidic environments, higher SOC values were recorded for both afforested sites, with mean values in the 0–10 cm layer of about 8.2% and 7.9% (managed and non-managed, respectively). Lower values were recorded in the non-managed dehesa systems (0.7%) (Figure 2).

Regarding the SOC/N ratio, significant differences ($p < 0.05$) were only found in the 0–10 and 20–30 cm layers (Table S1, Supplementary Materials). Higher values were found in the managed afforested sites in the topsoil and at depth in the non-managed dehesa. Lower values were observed in the managed dehesa sites (Figure 3 and Table S1, Supplementary Materials).

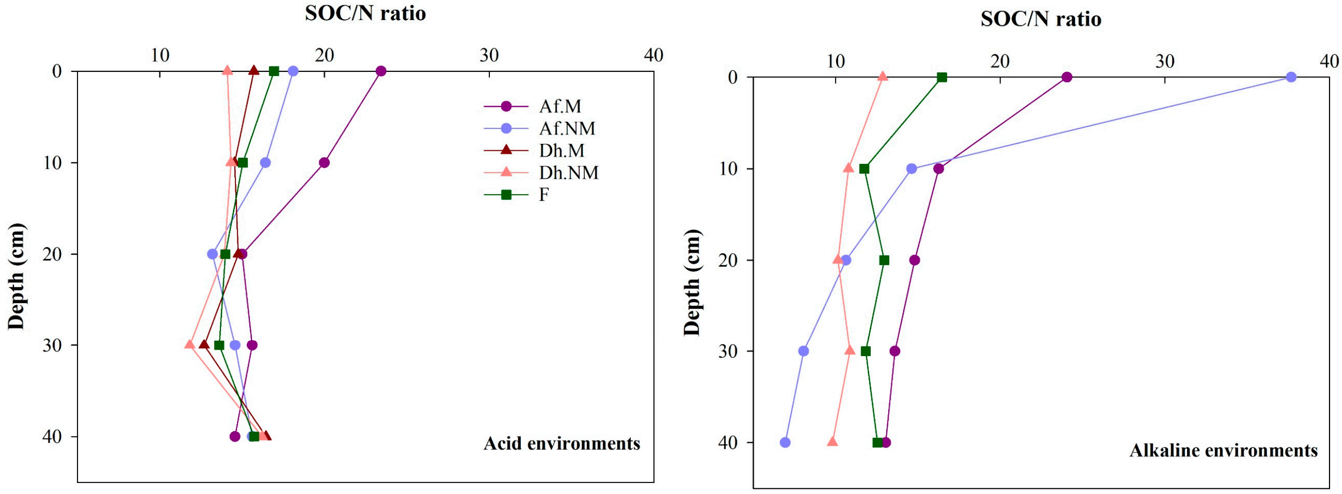

Figure 3. SOC/N ratio in the different LULC and depths in acidic and alkaline environments. Note: Af.M, managed afforestation; Af.NM, non-managed afforestation; Dh.M, managed dehesa; Dh.NM, non-managed dehesa; F, secondary forest.

Contrary to this, in alkaline environments, a higher SOC content in the 0–10 cm layer was recorded in the forest sites (7.7%) (Figure 2). At depth, the highest values were observed in the non-managed dehesa systems (2.6%). It should be also highlighted that no significant differences ($p < 0.05$) were observed between dehesa and afforested systems (Table S1 Supplementary Materials).

Related to SOC/N ratios, higher values were found in managed afforested sites. In deeper soils, lower values were recorded in the non-managed afforested sites (Figure 3).

3.2. SOC and N Stocks

In acidic environments, total SOC stocks ranged between 93.8 and 257.9 Mg C ha^{-1}, of which 31%–42% were stored in the first 10 cm (Figure 4). The highest SOC stocks were recorded in the managed afforested sites, and the lowest in the dehesa systems (significant differences at 0.05 level). In alkaline environments, total SOC stocks ranged between 116.0 and 181.7 Mg C ha^{-1}, of which 33%–44% were stored in the first 10 cm. No significant differences ($p < 0.05$) were recorded in alkaline environments (Figure 4). Significant differences ($p < 0.05$) were observed between SOC stocks from the same land use and cover but different soil environments for Af.M and Dh.NM.

Significant differences ($p < 0.05$) were observed in the nitrogen stocks in acidic environments (Figure 4). Nitrogen stocks ranged between 5.9 and 15.8 Mg N ha^{-1} (28.9%–42.8% in the first 10 cm). The lowest values were recorded for the dehesa systems and the highest for the afforested sites. In alkaline environments, the N stocks oscillated between 10.9 and 16.4 Mg N ha^{-1} (30.9%–45.9% in the first 10 cm). The highest values were recorded for both non-managed systems. In acidic environments, significant differences ($p < 0.05$) were mainly related to the LULCC, while in alkaline environments, significant differences ($p < 0.05$) were linked to the management of the afforested and dehesa sites. As occurred for SOC, significant differences ($p < 0.05$) were observed between N stocks from same land use and cover but different soil environments for Af.M and Dh.NM.

In order to determine the most important soil parameters for SOC accumulation, PCA was carried out. Data were grouped according to LULCs (Figure 5A) and environment (Figure 5B), and PCA scores were shown in both planes, PC1 and PC2. In the PC1 plane (46.5% of the explained variability), all of the parameters related with SOC and N content, physical and water-related properties were found (bulk density, saturation, etc.). In the PC2 plane (18.8%), data variability explanation was determined by texture (with silt and sand in the negative eigenvector and clay in the positive one), $CaCO_3$ content, pH and electrical conductivity properties.

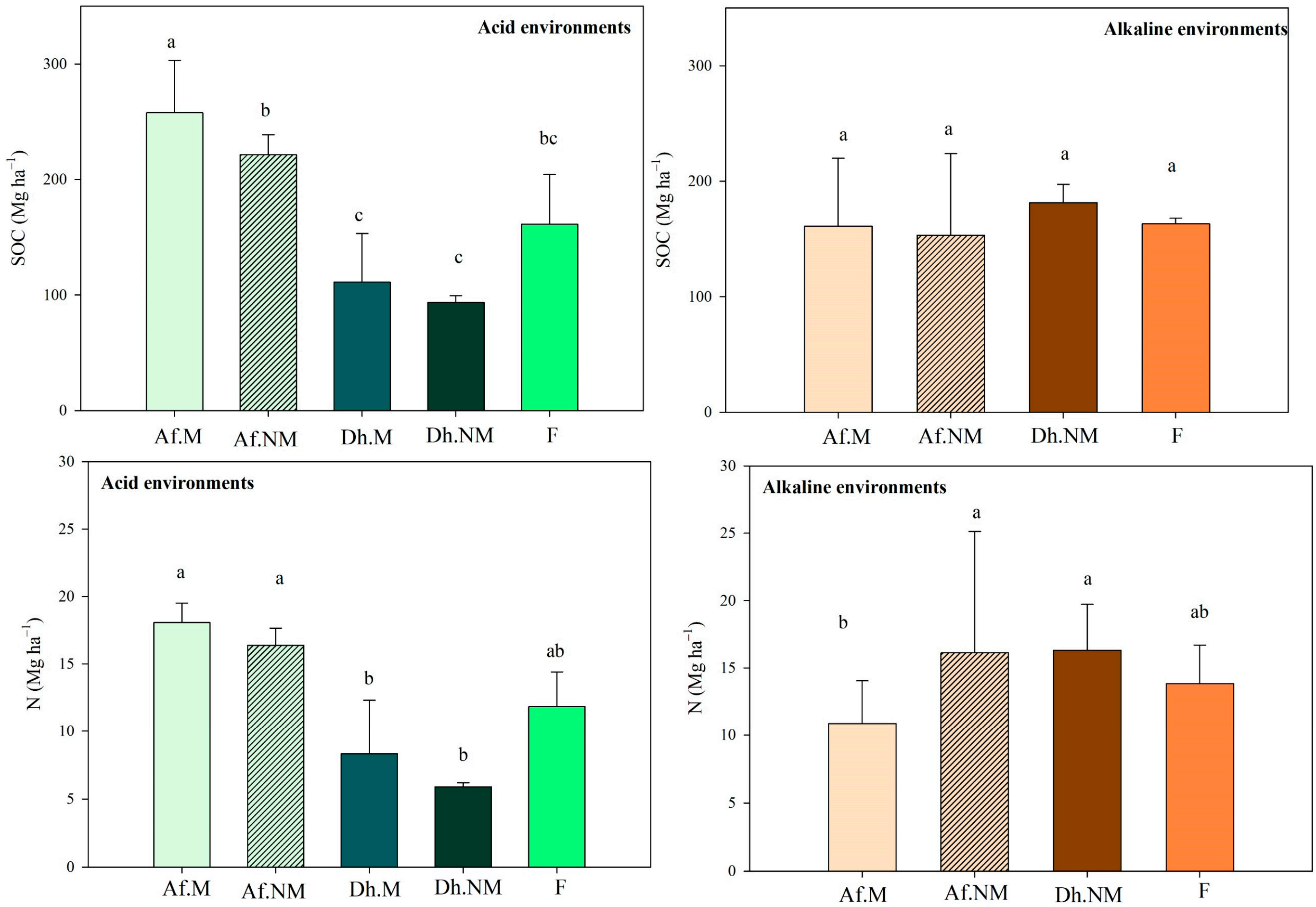

Figure 4. Soil organic carbon (SOC) and nitrogen (N) stocks and the different LULC in acidic and alkaline environments. Note: Af.M, managed afforestation; Af.NM, non-managed afforestation; Dh.M, managed dehesa; Dh.NM, non-managed dehesa; F, secondary forest. Different lower-case letters indicate significant differences (p-value < 0.05) between LULCs within the same graph.

It was possible to observe some differences between LULCs (Figure 5A), although not enough to make a strong distinction between them. F (situated on the negative side of PC2) was well differentiated from Af.NM and Dh.M (located on the positive side of PC2). Meanwhile, Af.M and Dh.NM were clustered at the center of both components and intersected with the rest of the LULCs.

Soil environment influence was also studied, and two groups can be differentiated (Figure 5B). Acidic soils were situated on the positive eigenvector of PC2, mainly influenced by clay content. Meanwhile, alkaline soils were clustered in the negative side of PC2, variating according to $CaCO_3$, sand, and silt content, and pH. This demonstrates the importance of considering the soil environment's influence in SOC studies.

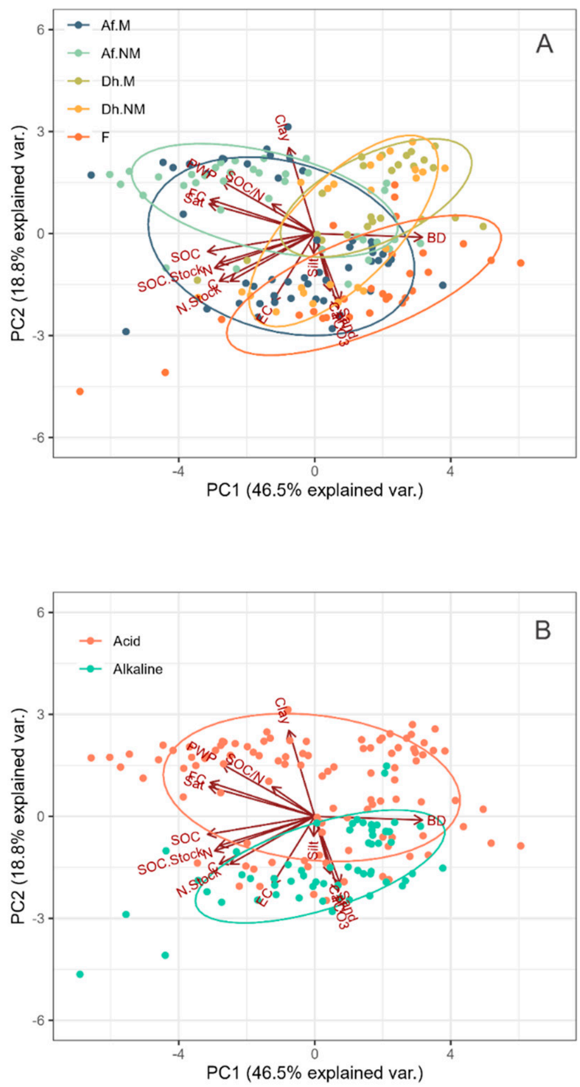

Figure 5. Principal component analysis (first, PC1, and second, PC2, components are represented) of the individual parameters differenced by LULC category (**A**) and soil environment (acidic or alkaline, (**B**)). Note: C, carbon content; N, nitrogen content; SOC, soil organic carbon content; SOC/N, soil organic carbon/nitrogen ratio; SOC.Stock, soil organic carbon stock; N.Stock, nitrogen stock; BD, bulk density; EC, electrical conductivity; FC, field capacity; Sat, saturated soil moisture; PWP, permanent wilting point; Af.M, managed afforestation; Af.NM, non-managed afforestation; Dh.M managed dehesa; Dh.NM, non-managed dehesa; F, secondary forest.

3.3. SOC Density Fractionation

In all LULCs, the contribution of the HF to the total SOC represents the most important part of the SOC (Figures 6 and 7). Table S2 in the Supplementary Materials indicates the significant differences ($p < 0.05$) in the percentage of the different fractions in the total SOC at depths of 0–20 and 20–40 cm. In acidic environments at 0–20 cm, significant differences ($p < 0.05$) were only found for the FLF between non-managed afforested sites, managed afforested sites, forest sites and non-managed dehesa sites. At 20–40 cm, significant differences ($p < 0.05$) were found for the FLF (between forest sites and both afforested sites) and for the HF (between managed afforested sites and non-managed afforested and dehesa sites, and between both afforested sites and forest sites). The contribution of the HF to SOC was slightly higher in both dehesa systems in the top layers, while the FLF was higher in the non-managed afforested sites. The lowest FLF contribution was recorded at all depths in the forest system (Figure 6).

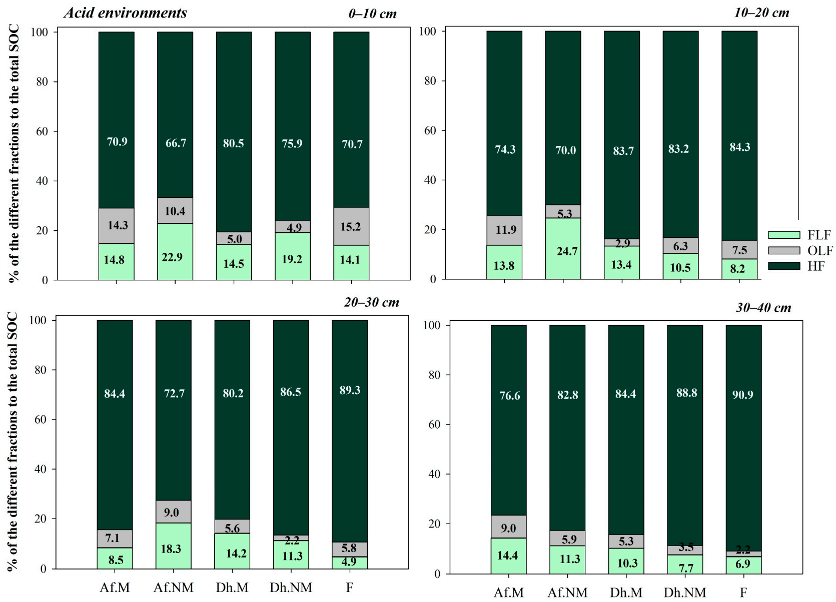

Figure 6. Contribution (%) of the different fractions to the total soil organic carbon (SOC) at the different depths in acidic soil environments. Note: FLF, free light fraction; OLF, occluded light fraction; HF, heavy fraction. Note: Af.M, managed afforestation; Af.NM, non-managed afforestation; Dh.M, managed dehesa; Dh.NM, non-managed dehesa; F, secondary forest.

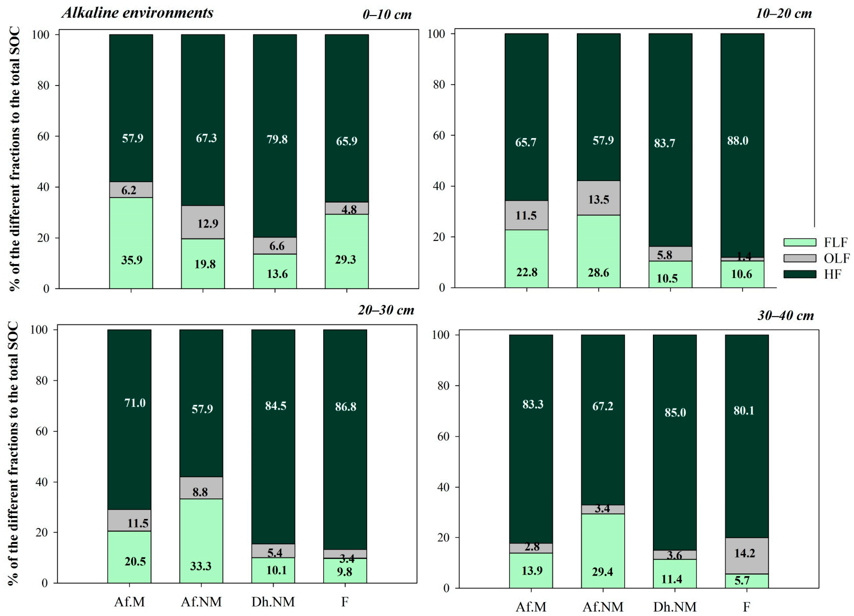

Figure 7. Contribution (%) of the different fractions to the total soil organic carbon (SOC) at the different depths in alkaline soil environments. Note: FLF, free light fraction; OLF, occluded light fraction; HF, heavy fraction. Note: Af.M, managed afforestation; Af.NM, non-managed afforestation; Dh.M, managed dehesa; Dh.NM, non-managed dehesa; F, secondary forest.

In alkaline environments, similar results were observed. The highest contributions of the HF and FLF were recorded for the dehesa and afforested systems, respectively. The lowest FLF contribution was recorded at all depths in the non-managed dehesa and forest systems (Figure 7). At 0–20 cm, significant differences ($p < 0.05$) were found only in the HF between both afforested sites and dehesa sites (Table S2 Supplementary Materials). At 20–40 cm, significant differences ($p < 0.05$) were found in the FLF and HF. In the FLF, differences were recorded between forest sites and non-managed systems, while for the HF, differences were also found between forest sites and non-managed systems.

Differences between acidic and alkaline environments were also observed for each soil type, finding significant differences (*p*-value < 0.05) in the surface horizon (0–20 cm) between the FLF fraction of Af.M and the OLF fraction of F. At a deeper horizon (20–40 cm), differences were found for the FLF in Af.NM.

SOC/N ratios of the HF tended to decrease with depth, whereas an opposite and irregular pattern was observed for the FLF and OLF (Figure 8), and in general, higher SOC/N ratios were found in the FLF and OLF compared to those recorded in HF, due to the organic composition of labile fraction. Table S2 in the Supplementary Materials indicates the significant differences ($p < 0.05$) in the SOC/N ratios between LULC at 0–20 and at 20–40 cm. In acidic environments, at 0–20 cm, significant differences ($p < 0.05$) were found in the FLF (between managed afforested sites and non-managed afforested sites and both dehesa sites) and in the HF (between managed afforested and forest sites and non-managed dehesa sites). At 20–40 cm, significant differences ($p < 0.05$) were recorded in the FLF (non-managed dehesa and forest and afforested sites and both managed forest systems) and the OLF (between managed afforested sites and both dehesas). In alkaline environments, significant differences ($p < 0.05$) were recorded at 0–20 cm only in the FLF (between non-managed dehesa and forest sites and both afforested sites). At 20–40 cm, significant differences ($p < 0.05$) were observed in the FLF (between non-managed afforested sites and forest and both dehesa sites and between managed afforested and non-managed dehesa sites). To sum up, significantly higher ($p < 0.05$) SOC/N ratios were recorded for the afforested sites in the topsoil, especially in alkaline environments, suggesting a lower quality of the organic matter and significant lower ($p < 0.05$) ratios in the dehesa systems.

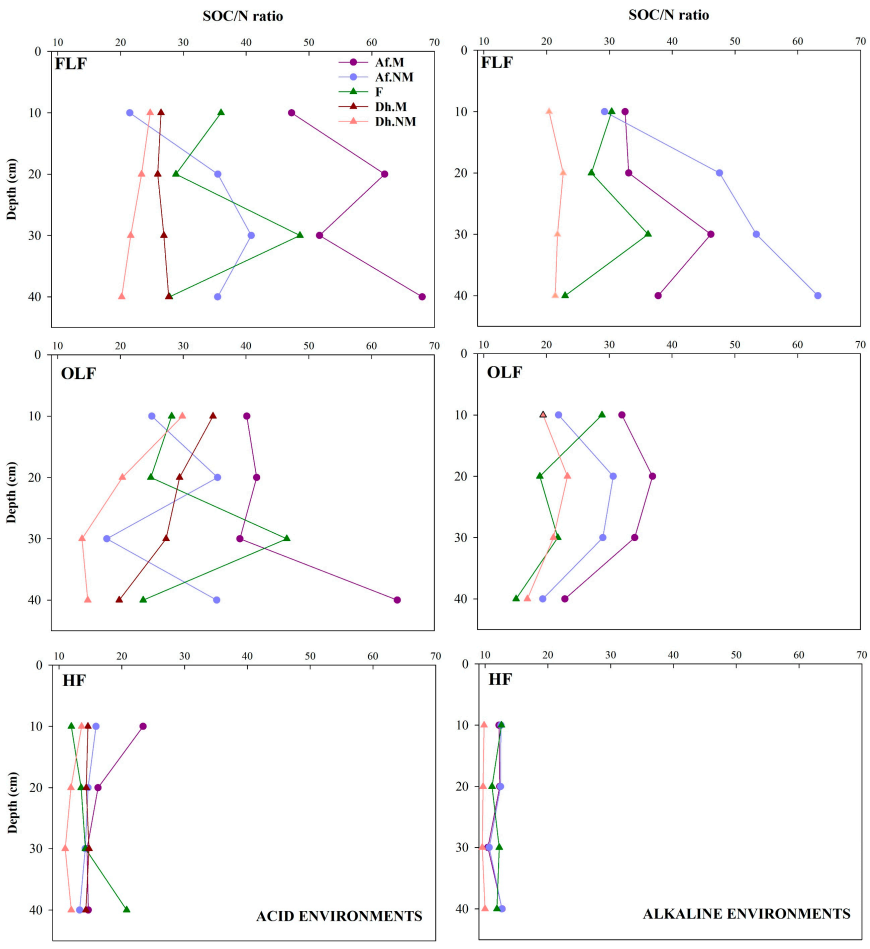

Figure 8. SOC/N ratios in the three different density fractions (FLF, free light fraction; OLF, occluded light fraction; HF, heavy fraction) at different depths in acidic and alkaline soil environments.

4. Discussion

This study addresses the question of how soil properties and SOC dynamics change under different forest systems in Mediterranean humid mountain areas. From the 1950s onwards, the process of land abandonment enhanced forest expansion, due to secondary succession or afforestation programs mainly with coniferous species, but also with the maintenance of traditional silvo-pastoral systems (dehesas) close to villages. Hence, comprehending soil dynamics in Mediterranean forest systems is crucial for making appropriate land management decisions.

Numerous studies conducted worldwide have shown that land use and land cover changes and forest systems significantly impact soil properties, soil quality, and SOC accumulation. This holds particularly true for the Mediterranean region [36,37]. However, there is no agreement in the literature on the effect of forest management on soil properties, nor in relation to the effects of afforestation and managed systems in SOC [38]. Likewise, in Mediterranean mountain areas, these processes have been widely described in alkaline soils; however, less information is known about what happens in acidic soil environments [37,39].

Our results suggest that in acidic soils, afforestation and management of these fast-growing tree species (*P. sylvestris*) (i.e., thinning and clear-cutting harvesting, extensive livestock cultivation) in the best locations have a immediately significant effect ($p < 0.05$) on soil properties and SOC accumulation. These results were also confirmed in other Mediterranean areas [40,41]. Afforestation with conifers tends to accumulate more organic matter on the forest floor (see Figure 2). In all cases, SOC concentration decreased with increasing depth of the mineral soil. In acidic environments, SOC contents were significantly higher ($p < 0.05$) under conifers than under oak trees in the complete soil profile. However, no clear pattern was observed in alkaline soils. Romanyà and Rovira [42] found similar results, with higher SOC accumulation under pines a few decades after afforestation in acidic soils, this being faster than the SOC increase generally reported for alkaline soils. This suggests a rapid SOC recovery in acidic soils.

Marked differences were observed in the results between acidic and alkaline environments, but these differences can also be explained by the altitude, vegetation species, and history of land use and land covers. In this sense, one of the factors that affected these dynamics may be the previous land use and land cover in the afforested sites: pastures and croplands in acidic and alkaline, respectively. Pastures display high SOC stocks and high root density in the topsoil; in contrast, croplands are more depleted in SOC due to agricultural activities, and it takes longer until high SOC values occur in former arable soils [40].

Likewise, tree species influence SOC stocks through the quantity of organic matter input from litterfall and root activity. Tree species can exert significant effects on SOC stocks compared to other management interventions. Chiti et al. [16] characterized SOC in forest systems in Spain, and they found that conifers store a significantly higher amount of SOC than broadleaved forests. In comparison to other forest ecosystems in Mediterranean areas, the SOC storage in dehesas is relatively low [32,43], due to the long-term intensive land occupation and exploitation with the creation of open woodlands. Likewise, no significant differences ($p < 0.05$) were found in dehesa systems compared to the managed (grazed) and non-managed sites in acidic environments. Ganatsas et al. [44] found similar values in Greek forests and suggested that these values correspond to forests that have undergone great pressures in the past, such as overgrazing or wood exploitation. Livestock grazing, for example, results in the removal of a great amount of plant material, diminishing the available litter for decomposition and reducing the organic input into the soil. These forest systems were highly degraded in the past due to agricultural activities, but have been slowly restored/managed. Likewise, similarly to other studies [45], SOC stocks of secondary forests are slightly higher than those of old open forest systems in both environments.

The fractionation results also revealed differences between coniferous and broadleaved systems which could have important implications. The high SOC accumulation in afforested sites was mainly caused by the high contribution of labile fractions (especially the FLF). In this sense, the greater accumulation of SOC in forests comprising coniferous species in comparison to those comprising deciduous species, especially in acidic environments, can be attributed to slower decomposition, which in turns is a consequence of the different litter quality and soil functioning between forest systems. Therefore, the smaller SOC/N ratios reflect that the organic matter degradation in the old open forest (dehesa) occurs faster than in the afforestation sites (conifers stands) because microorganisms prefer the digestion of litter with a low SOC/N ratio (lower than 20) to satisfy their nitrogen needs [46]. Also, Vazquez et al. [17] showed that the SOC/N ratio of Cork oak soil is considerably lower when compared to pine plantation soil, suggesting that the relative enrichment in labile N forms may explain the higher decomposability of *Quercus* sp. litter inputs. The SOC/ratio of the different fractions followed the same trend as bulk soils in most of the cases, with higher values in afforested sites than in broadleaved sites. Likewise, the ratio was higher in the FLF than in the OLF and HF.

At this point, it is interesting to refer to the saturation theory of carbon accumulation in the system [47,48], according to which the stabilization of SOC in the soil depends on four pools: the protected carbon pool in the soil microaggregates; the protected pool in the silt and clay particles; the biochemically protected pool; and the unprotected carbon pool. This might lead us to suspect that acidic pine afforestation could cause a state of "over-saturation" in the system (more input than the system can assimilate), where the acidic environment and the biochemical nature of the litter might partially stabilize carbon in interactions with Al [49], locking its dynamics within a mineralized state (HF). Although it is of great interest to calculate the saturation of the system to develop a better understanding of the processes that transcend these results, the methodologies established in the literature for its analysis [50–52] differ from that followed in this study. As the lack of information makes it impossible to study the saturation state from a quantitative perspective, this leaves the door open to the need for its evaluation in future research.

Likewise, the effects of forest management on SOC dynamics are still not clear. Our results only show significantly higher ($p < 0.05$) SOC values in managed acidic afforestation sites compared to non-managed sites. Also, slightly higher values were observed in the other managed sites. Zhang et al. [53] concluded that light thinning increases SOC stocks by 17%. However, other studies noted that forest thinning and clear-cutting harvesting may have a slightly negative impact on SOC stocks, due to the reduction in biomass and carbon inputs, while also changing the microclimate [2,54]. The importance and effects of the different management systems should be highlighted, as well as how the wood and material are used after different forest maintenance activities.

The importance of the soil environment (acidic or alkaline) for both the SOC stock and its subsequent mineralization has thus been demonstrated. This is influenced by the nature of the litter depending on the tree species, as well as the type of past and present management [16,39,42]. This could justify why, while in a first analysis of acidic environments, we find a higher stock in afforestation sites (possible carbon saturation of the system due to the nature of the litter and the physical-chemical nature of the soil) [48,49], in alkaline soils (where the degradation of organic matter is not blocked by the acidity of the soil), the greatest contribution of carbon is produced in growing forests with a significant presence of undergrowth, at least in the first horizons [55,56]. Likewise, the management of afforestation and dehesas leads to a greater contribution of organic matter and debris, which in the case of pine trees will be more difficult for soil organisms to digest than deciduous species [18,57], increasing the stock level. However, when observing the fractionation data, it can be seen that, in general, afforestation reaches a lower percentage of HF than forests and dehesas of deciduous species, being less profitable as a long-term carbon sequestration tool. It can be deduced from this that the higher acidity of the environment can lead to differences between LULCs in the first stage of carbon stabilization (possible saturation of the system in afforestation) [48], which will not be reflected in alkaline soils as they have fewer limitations in their physico-chemical nature.

Finally, this study validated the fact that it is crucial to understand and quantify changes in the different SOC fractions, and not only SOC accumulation. Although the main changes occurred in the topsoil, changes were also observed in the deepest layers in all of the SOC fractions, suggesting the need to include this information in SOC research.

5. Conclusions

In conclusion, this study confirms our hypothesis, indicating that afforestation with pine induced profound changes in the soil characteristics and SOC accumulation, significantly increasing ($p < 0.05$) the size of the SOC and N pools compared to other forest ecosystems, especially in acidic environments. The higher SOC accumulation under afforestation than in dehesa systems (257.9 Mg C ha^{-1} and 93.8 Mg C ha^{-1}, respectively) revealed rapid SOC accumulation, especially in acidic environments. Broadleaved forests seem to be most beneficial in terms of the sequestration of stable mineral SOC in the forest (generally over 80% of HF in all depths), whereas conifers are expected to achieve higher SOC stocks under less favorable conditions.

The higher SOC accumulation in afforested sites was caused mainly by the high contribution of labile fractions (between 8.5 and 35.9% FLF), which together with the high SOC/N ratios can be interpreted as causing slower organic matter stabilization under conifers. In this sense, the higher SOC accumulation in afforested sites does not mean stabilization in the long-term, due to the high accumulation of labile unprotected soil organic carbon.

This study corroborated that it is crucial to understand and quantify changes in the different SOC pools, and not only estimate SOC accumulation in forest systems. Our results confirm that although total carbon concentrations increased under the afforestation system with conifers, this SOC is stored in less stable carbon pools (FLF) compared to broadleaved forest systems. Likewise, marked differences were observed in the results between acidic and alkaline environments and the history of land use and land covers. Finally, the management system is a significant factor, especially in afforested sites.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15010166/s1, Table S1: Land use and land cover, soil environments (acid or alkaline) and depth values of physical and chemical soil properties (means values with standard deviation and significant differences); Table S2: Statistic and resulting *p*-value of the Kruskal-Wallis one-way analysis of variance of the fractionation results over the different Land Use and Land Covers (LULC), consisting of 4 replicates per LULC and depth. Samples were grouped by depths: 0–20 and 20–40 cm.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the fact that they are currently being used as part of a major project.

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